Primerdesign

R01006

Joint Infection Multiplex Kit

Kit version: 1

Target regions:

Cutibacterium: rpsD & purB genes

Staphylococcus aureus: PRK14902 gene region

genesig®PLEX Kit

100 tests

GENESIG

Kits by Primerdesign

For general laboratory and research use only

Introduction

Cutibacterium

This genesig®PLEX detection kit targets the rpsD and purB genes from Cutibacterium (formerly known as Propionibacterium). Cutibacterium are Gram-positive, slow growing cutaneous anaerobic commensals that have been reported in prosthetic joint infections. Their genomes are approximately 2.5 Mb in length and there are five species within the genus: Cutibacterium acnes, Cutibacterium granulosum, Cutibacterium avidum, Cutibacterium modestum and Cutibacterium namnetense. The key species responsible for infections in joints are the former four, with C. acnes responsible in most cases.

Diagnosing Cutibacterium for treatment has proved challenging as typical signs of infection are absent, often with the only symptom being pain. Due to the slow growth rate, microbial cultivation diagnoses are often inconclusive as they are usually only several days in duration, whereas Cutibacterium typically requires at least a 14-day cultivation time. Molecular detection is therefore the preferred methodology to provide a more rapid diagnosis.

Staphylococcus aureus

This genesig®PLEX detection kit targets the PRK14902 gene region of Staphylococcus aureus (S. aureus). S. aureus is a spherical, Gram-positive bacterium of the Staphylococcaceae family. The genome of this species is around 2.8Mb in length. Virulence factors for S. aureus are encoded by phages, pathogenicity islands, staphylococcus cassette chromosome and plasmids, which contain genes for antibiotic resistance such as those found in Methicillin Resistant Staphylococcus aureus (MRSA).

S. aureus can be found on the skin and mucosal membranes of healthy individuals, but any abrasion to these barriers allows the bacterium to enter the wound, colonise and cause infection. As this bacterium lives on the skin, it can be transmitted easily between individuals and is commonly seen in hospital acquired infections.

S. aureus can cause a variety of different infections ranging from mild skin infections such as impetigo to invasive and toxic mediated diseases, and is a common cause of bone and joint infections.

Specificity

The genesig®PLEX kit is designed for the in vitro detection of Cutibacterium acnes, Cutibacterium avidum, Cutibacterium modestum, Cutibacterium granulosum and Staphylococcus aureus. The assays within this kit are predicted to detect over 95% of sequences available on the NCBI database since the date of last review.

The dynamics of genetic variation means that new sequence information may become available after the initial design. If you require further information or have a specific question about the detection profile of this kit, then please send an email to techsupport@primerdesign.co.uk and our team will answer your question.

Kit contents

Quantity	Component	Tube	Cap Colour
2	**Joint Infection primer/probe mix** (50 reactions) Cutibacterium: FAM S.aureus: VIC Internal extraction control: Cy5		**BROWN**
1	**Joint Infection positive control template**		**RED** (in silver foil wrapper)
2	**oasig® Lyophilised 2X qPCR Master Mix** (50 reactions per glass vial)		**SILVER**
2	**oasig® Master Mix resuspension buffer**		**BLUE**
1	**genesig® Easy DNA internal extraction control**		**BLUE** (in gold foil wrapper)
1	**Template preparation buffer** for resuspension of internal control template and positive control template		**YELLOW**
1	**RNase/DNase free water** for resuspension of the primer/probe mix		**WHITE**

Reagents and equipment to be supplied by the user

Real-time PCR Instrument
Must be able to read fluorescence through FAM, VIC/HEX and Cy5.

Extraction kit
This kit is recommended for use with genesig® Easy DNA/RNA extraction kit or exsig®Mag. However, it is designed to work well with all processes that yield high-quality nucleic acid with minimal PCR inhibitors.

Pipettors and filter tips

Vortex and centrifuge

1.5 ml microtubes

qPCR plates or reaction tubes

Kit storage and stability

This kit is stable for shipping at ambient temperature but should be stored at -20°C upon arrival. Once the lyophilised components have been resuspended, they should not be exposed to temperatures above -20°C for longer than 30 minutes at a time and unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Primer Design Ltd does not recommend using the kit after the expiry date stated on the pack.

Suitable sample material

This kit can be used with all types of samples from various origins. Please ensure that the extracted nucleic acid samples are suitable in terms of purity, concentration, and DNA integrity. An internal extraction control is provided to test for non-specific PCR inhibitors.

Dynamic range of test

Under optimal PCR conditions the kit can achieve priming efficiencies between 90-110% and detect less than 100 copies of target template.

Principles of the test

Real-time PCR

Individual primer and probes designed for each pathogen and the internal extraction control have been combined into a single reaction and these can be detected through the different fluorescent channels as described in the kit contents.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the target DNA. Fluorogenic probes are included in the same reaction mixture which consists of a DNA probe labelled with a 5`-dye and a 3`-quencher. During PCR amplification, the probe is cleaved, and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of qPCR platforms.

Positive control

The kit contains a single positive control that contains templates for the two targets in the test. Cutibacterium will give a signal in the FAM channel, and S. aureus will give a signal in the VIC/HEX channel. Each time the kit is used, at least one positive control reaction must be included in the run.

A positive result indicates that the primers and probes for detecting each target are working properly in that particular run. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false positive results. This can be achieved by handling these components in a post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control

To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNase/DNase free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. It is also known as a No Template Control or NTC.

Cutibacterium DNA is known to be highly prevalent in the air and environment. The negative control may therefore give a late positive signal due to contamination. See the ‘Interpretation of Results’ section of the handbook for guidance on results interpretation where environmental contamination is evident.

Internal DNA extraction control

When performing DNA extraction, it is often advantageous to have an exogenous source of DNA template that is spiked into the lysis buffer. This control DNA is then co-purified with the sample DNA and can be detected as a positive control for the extraction process. Successful co-purification and qPCR for the control DNA also indicates that PCR inhibitors are not present at a high concentration.

The primers and probe necessary to detect the internal extraction control are included in the multiplex primer and probe mix. The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quality and quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28 ±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Resuspension protocol

To minimise the risk of contamination with foreign DNA, we recommend that all pipetting is performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. **Pulse-spin each tube in a centrifuge before opening.**
   This will ensure lyophilised primer probe mix or template is in the base of the tube and is not lost upon opening the tube.

2. **Resuspend the primer/probe mix in the RNase/DNase free water supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in water	Volume
Pre-PCR pack	
Joint Infection primer/probe mix (**BROWN**)	55 µl

3. **Resuspend the internal control template and positive control template in the template preparation buffer supplied, according to the table below:**
   To ensure complete resuspension, vortex the tube thoroughly.

Component – resuspend in template preparation buffer	Volume
Pre-PCR heat-sealed foil	
Internal extraction control DNA (**BLUE**)	500 µl
Post-PCR heat-sealed foil	
Joint Infection positive control template (**RED**)*	500 µl

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

4. **Resuspend the lyophilised Master Mix in oasig resuspension buffer, according to the table below:**

Component – resuspend in oasig resuspension buffer	Volume
oasig lyophilised 2X qPCR Master Mix (SILVER)	525µl

DNA extraction

The internal extraction control DNA can be added to either lysis/extraction buffer or to the sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control DNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. **Add 4µl of the Internal extraction control DNA (BLUE) to each sample in DNA lysis/extraction buffer per sample.**

2. **Complete DNA extraction according to the manufacturer's recommended protocols.**

qPCR detection protocol

For optimum performance and sensitivity, all pipetting steps and experimental plate set up should be performed on ice. After the plate is prepared proceed immediately to the amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. **For each DNA sample prepare a reaction mix according to the table below:**
   Include sufficient reactions for positive and negative controls.

Component	Volume
oasig® lyophilised 2X qPCR Master Mix (SILVER)	10 µl
Joint Infection primer/probe mix (**BROWN**)	1 µl
RNase/DNase free water (WHITE)	4 µl
Final Volume	**15 µl**

2. **Pipette 15µl of this mix into each well according to your qPCR experimental plate set up.**

3. **Pipette 5µl of DNA sample into each well according to your experimental plate set up.**
   For negative control wells use 5µl of RNase/DNase free water. The final volume in each well is 20µl.

4. **Pipette 5µl of positive control template into each well according to your plate set up.**
   The positive control contains templates for Cutibacterium and S.aureus. The final volume in each well is 20µl.

qPCR Amplification Protocol

Recommended amplification conditions when using oasig® Lyophilised qPCR Master Mix.

	Step	Time	Temp
	Enzyme activation	2 mins	95°C
Cycling x 50	Denaturation	10 secs	95°C
	DATA COLLECTION*	60 secs	60°C

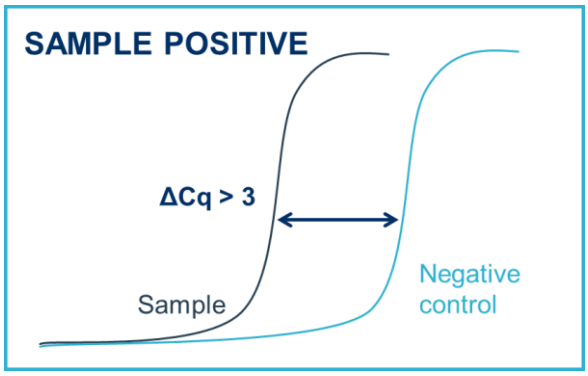
* Fluorogenic data should be collected during this step through the FAM, VIC/HEX and Cy5 channels.

Interpretation of results

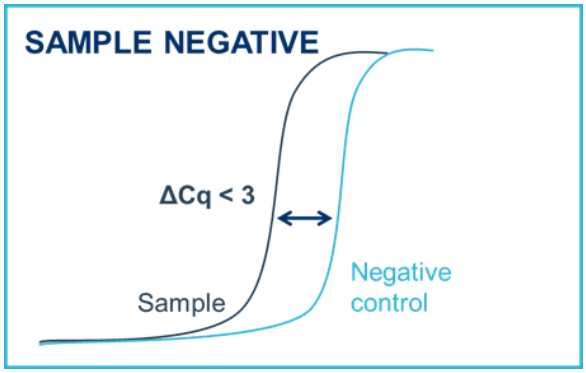
Target (FAM, VIC/HEX)	Internal extraction control (Cy5)	Positive Control	Negative Control	Interpretation
FAM +	**+ / -**	**+**	**-**	**Cutibacterium POSITIVE RESULT**
VIC/HEX +	**+ / -**	**+**	**-**	**S. aureus POSITIVE RESULT**
-	**+**	**+**	**-**	**NEGATIVE RESULT**

+ / -	**+ / -**	**+**	**≤30**	**EXPERIMENT FAILED** Due to test contamination
+ / -	**+ / -**	**+**	**>30**	*****
-	**-**	**+**	**-**	**SAMPLE PREPARATION FAILED**
+ / -	**+ / -**	**-**	**+ / -**	**EXPERIMENT FAILED**

*Where the test sample is positive and the negative control is positive with a Cq > 30, the sample must be reinterpreted based on the relative signal strength of the two results:



If the sample amplifies > 3 Cq earlier than the negative control, then the sample should be reinterpreted (via the table above) with the negative control verified as negative.



If the sample amplifies < 3 Cq earlier than the negative control Cq, then the positive sample result is invalidated, and a negative call is the correct result.

Positive control

The positive control well should give a signal and amplification plot for Cutibacterium through the FAM channel and for S. aureus through the VIC/HEX channel. There is no Internal control template within the positive control so the Cy5 channel should give no signal (flat amplification plot). The positive control signals indicate that the kit is working correctly to detect each target.

The positive control is expected to amplify between Cq 16 and 23. Failure to satisfy this quality control criterion is a strong indication that the experiment has been compromised.

Negative control

The negative control is generally expected to be free of amplification. However, Cutibacterium DNA is known to be highly prevalent in the air and environment. The negative control may therefore give a late positive signal in the FAM channel due to contamination. Follow the guidance on the previous page for interpretation.

Internal DNA extraction control

The Cq value obtained with the internal control will vary significantly depending on the extraction efficiency, the quality and quantity of DNA added to the PCR reaction and the individual machine settings. Cq values of 28±3 are within the normal range. When amplifying a target sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

Sample data

Presence of the targets are detected in the channels indicated in the kit contents section. Positive signals indicate positive tests for those targets. It may be possible for samples to contain multiple targets, therefore positive results in the FAM and VIC/HEX channels may be present.

Notices and disclaimers

This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Primer Design Ltd genesig® detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer's recommendations the right to claim under guarantee is expired.

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